Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE INFLUENCE OF CHLORAL HYDRATE ON SERUM ANAPHYLAXIS.*†

EDWIN J. BANZHAF AND L. W. FAMULENER.

(From the Research Laboratory of the Department of Health, New York City,
William H. Park, M.D., Director.)

IN a preliminary communication read before the Society for Experimental Biology and Medicine in February 1908, we reported the following:

We found that by injecting a solution of chloral hydrate which was just sufficient in strength to produce hypnosis, fully 75 per cent of all serum-sensitized guinea-pigs were completely protected from a second injection of serum into the peritoneal cavity, whereas 90 per cent of controls died. We believed that with improved technic in the dosage of chloral hydrate it would be possible to protect 90 per cent of all fully sensitized guinea-pigs. By "fully sensitized," we meant that at least three weeks' or a month's time should have elapsed before the second injection of serum into guinea-pigs which had received horse serum alone (1/100 to 1/500 c.c.); at least seven or eight weeks should have elapsed before the second injection. By allowing the above interval of time to elapse, over 90 per cent of our controls died within an hour, most of them within 20 minutes.

We found that the dose of the chloral hydrate per gram weight of the animal was not a simple ratio, no fixt amount could be stated, much depending upon the individual idiosyncrasy of the animal. Approximately 75 milligrams of the drug to a 250 gram guinea-pig, and 100 milligrams to a 300 gram guinea-pig produce the degree of hypnosis desired.

We used a fresh 10 per cent solution of chloral hydrate, carefully measured out the required amount into a small sterile beaker, and added an equal amount of sterile water. This diluted solution was injected into the muscles of the thigh of the animal, half into one leg and half into the other. After 20 to 30 minutes, the needle was inserted into the peritoneal cavity. This caused muscular twitching and slight movement of the head. This indicated the proper degree of hypnosis. The injection of 5 c.c. serum was then given and the animal kept in a warm room. No symptoms appeared and the sleep was undisturbed. After 2½ to 3½ hours have elapsed, the animal slowly recovers from the effect of the drug. No symptoms or ill effects have been observed in any of the animals. Observations have been followed for over two weeks after treatment. The animal, after the effects of the drug have disappeared, will react with characteristic symptoms of anaphylaxis if given a third injection of the serum. We injected only 24, 48, and 72 hours after recovery from the effects of the drug.

If the dose of chloral hydrate has not been sufficient, the insertion of the needle into the peritoneal cavity will cause marked muscular movements, raising of the head, and an attempt to regain its feet. Under these conditions if the serum is injected the animal will die of anaphylaxis.

---

* Received for publication March 7, 1910.

† Read before the American Society of Biological Chemists, Baltimore, December, 1908.

On the other hand, if the animal shows no muscular twitchings whatever, the dose of chloral has probably been too large. We wish to emphasize the fact that great care must be used not to overdose the sensitized guinea-pig with chloral hydrate, altho a sensitized as well as a normal guinea-pig will recover from a large dose, considerably more than the amount mentioned above. Apparently the combined effects of the drug and the serum in a sensitized animal produce a deeper hypnosis than the drug when given alone.

Further work, following along the lines of the above preliminary report, has fully confirmed our earlier observations.

We realized that for our work it was essential to determine the best method for sensitizing the guinea-pigs so that on receiving the second injection of horse serum intraperitoneally the animals would surely die from anaphylaxis. We therefore injected several series of guinea-pigs subcutaneously with from 0.01 c.c. to 0.001 c.c. of horse serum. After 3 to 6 weeks, we found that in none of the series did 100 per cent of deaths occur when the animals were injected with 5 c.c. horse serum intraperitoneally. In our later work, we have used only those guinea-pigs which had survived the routine testing of antitoxin. After a lapse of three weeks or longer, these animals, in our experience, succumb almost without exception to the second injection of 5 c.c. horse serum intraperitoneally.

In this later work, we have given the second injection of serum only to those sensitized guinea-pigs which showed favorable hypnosis, indicated by only slight muscular twitchings when the needle is inserted into the peritoneal cavity. If muscular movements are marked we do not inject. In this case, we allow the animal to recover from the effects of the drug, and next day increase the dose about one-fifth. This usually brings about the optimum hypnosis in about 25 to 30 minutes. In following this method we have frequently protected 100 per cent, while 100 per cent of the controls died.

In our work thus far it appears as tho the protection chloral hydrate affords a sensitized guinea-pig depends on the degree of sensitization. If we accept the hypothesis of Vaughan, this may be expressed as the amount of ferment or zymogen present in the sensitized guinea-pig, its supposed degree of activity or power in freeing or splitting off definite quantities of a poisonous substance from the serum given in the second injection accounting for the symptoms of anaphylaxis.

This appears to be the case in the following table. The eight guinea-pigs were well under the influence of the drug when the second injection was given. Guinea-pigs 393, 702, and 146 died in 15, 30, and 20 minutes respectively with typical symptoms of anaphylaxis, the heart continuing to beat after respiration ceased.

TABLE 1.

INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE FOLLOWED BY 5 C.C. NORMAL HORSE SERUM.

G.-P. No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate in Mg.	Second Injection Intraperitoneally	Symptoms	Results
330....	310	0.56 c.c. toxin+ 1/500 c.c. antitoxic horse serum	42	125	5 c.c. normal horse serum	None	Recovered
393....	265	" "	42	90	"	Typical	Death within 15 min.
355....	260	0.56 c.c. toxin+ 1/300 c.c. antitoxic horse serum	28	90	"	None	Recovered
376....	260	" "	28	85	"	"	"
358....	260	0.56 c.c. toxin+ 1/350 c.c. antitoxic horse serum	32	85	"	"	"
363....	280	" "	32	100	"	"	"
702....	395	0.55 c.c. toxin+ 1/300 c.c. antitoxic horse serum	62	180	"	Typical	Death within 30 min.
146....	335	" "	62	150	"	"	Death within 20 min.
313....	295	0.56 c.c. toxin+ 1/500 c.c. antitoxin horse serum	42		"	"	Death within 18 min.
372....	260	" "	42		"	"	Death within 25 min.
595....	240	0.56 c.c. toxin+ 1/200 c.c. antitoxic horse serum	28		"	"	Death within 12 min.
696....	240	" "	28		"	"	Death within 17 min.
513....	230	0.56 c.c. toxin+ 1/300 c.c. antitoxic horse serum	33		"	"	Death within 12 min.
347....	260	" "	33			"	Death within 33 min.

Many series of sensitized guinea-pigs were given chloral hydrate intramuscularly followed by the second injection of horse serum intraperitoneally, and in almost all cases one or two guinea-pigs of each series would die with typical symptoms of anaphylaxis, even tho the degree of hypnosis seemed sufficient. This leads us to believe that in such guinea-pigs more poison was elaborated or split off from the serum in a given time than in those that were protected; for example say, if just a single fatal dose of poison is split off, the chloral will protect, but if two or more fatal doses are split off, the chloral cannot protect the animal. A quantitative relationship may exist.

In Table 2 the controls died promptly, while those which received the chloral were protected.

Guinea-pigs 60 and 202 had received 180 and 160 milligrams chloral, respectively, the day before. They then weighed 410 and 370 grams. This amount, however, proved insufficient to produce the desired hypnosis. They were allowed to recover, and the following day weighed 370 and 335 grams respectively. They were again given the same amount of the drug, 180 and 150 milligrams. In 25 minutes favorable hypnosis was produced. The 5 c.c. of horse serum were then injected. The following day these two guinea-pigs were chloroformed and autopsied. No definite lesions were found.

TABLE 2.

INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE FOLLOWED BY 5 C.C. NORMAL HORSE SERUM.

G.-P. No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate in Mg.	Second Injection Intraperitoneally	Symptoms	Results
60....	370	0.56 c.c. toxin+ 1/350 c.c. antitoxic horse serum	85	180	5 c.c. normal horse serum	Slight twitching of ears	Recovered
202....	335	0.56 c.c. toxin+ 1/750 c.c. antitoxic horse serum	82	160	"	None	"
240....	370	0.56 c.c. toxin+ 1/400 c.c. antitoxic horse serum	74	170	"	"	"
777....	325	0.56 c.c. toxin+ 1/800 c.c. antitoxic horse serum	68	130	"	Slight twitching of ears	"
748....	345	0.56 c.c. toxin+ 1/250 c.c. antitoxic horse serum	68	155	"	"	"
438....	375	0.56 c.c. toxin+ 1/200 c.c. antitoxic horse serum	85		"	Typical	Death within 20 min.
143....	360	0.56 c.c. toxin+ 1/300 c.c. antitoxic horse serum	82		"	"	Death within 13 min.
251....	395	0.56 c.c. toxin+ 1/250 c.c. antitoxic horse serum	81		"	"	Death within 20 min.
256....	360	0.56 c.c. toxin+ 1/750 c.c. antitoxic horse serum	78		"	"	Death within 32 min.
25....	290	0.56 c.c. toxin+ 1/900 c.c. antitoxic horse serum	50		"	"	Death within 25 min.

Guinea-pigs 240, 777, and 748, three days later, again received 5 c.c. horse serum intraperitoneally; slight but definite symptoms appeared in the first two animals after about 15 minutes. These symptoms subsided in about 30 minutes. In the third animal questionable symptoms were noted. Other sensitized guinea-pigs that had been protected from the second injection of serum with chloral were also reinjected; about 75 per cent showed slight but definite symptoms.

CHLORAL HYDRATE INJECTED INTRAMUSCULARLY, FOLLOWED BY NORMAL HORSE SERUM INTRACARDIACALLY.

Sensitized guinea-pigs were given chloral hydrate in the same manner as mentioned above. Those animals which showed favorable hypnosis were injected directly into the heart with 0.25 c.c. of normal horse serum. After about one-half minute all the animals showed typical symptoms of anaphylaxis, dying within three to four minutes. All the controls also died within three to four minutes. This experiment was repeated several times with the same results; none of the guinea-pigs were protected.

All these animals were autopsied. We found no deaths due to direct hemorrhage. Gross lesions were found in other organs.

TABLE 3.

INTRAMUSCULAR INJECTIONS OF CHLORAL HYDRATE FOLLOWED BY NORMAL HORSE SERUM INTRACARDIACALLY.

G.-P. No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate in Mg. Intramuscularly	Second Injection Horse Serum Intracardiacally	Symptoms	Result	Autopsy
353	290	0.56 c.c. toxin+ 1/300 c.c. antitoxic horse serum	21	125	0.25 c.c.	Typical	Death within 3 min.	No hemorrhage in pericardium
535	330	0.56 c.c. toxin+ 1/400 c.c. antitoxic horse serum	51	160	"	"	Death within 4 min.	" "
107	300	"	20	140	"	"	"	"
237	280	"	20	120	"	"	"	"
218	520	0.56 c.c. toxin+ 1/100 c.c. antitoxic horse serum	29		"	"	Death within 3 min.	"
272	300	0.56 c.c. toxin+ 1/400 c.c. antitoxic horse serum	20		"	"	"	"
100	275	"	20		"	"	Death within 4 min.	"

In seeking an explanation for these results, we came to the conclusion that a certain optimum content of the drug in the circulation was essential to protect the animal; that possibly the lipoids played an important rôle in anaphylaxis; that if we injected a sufficient amount of chloral hydrate directly into the circulation, we would obtain a loose physico-chemical combination with the vitally important lipoids of the cells and in so doing change their normal relationship to the other cell constituents, through which an inhibition of the entire cell chemism would result. With this in mind, we injected sensitized guinea-pigs weighing between 275 and 300 grams with 30 milligrams of the drug directly into the heart. Even before completion of the injection, the animals were under the influence of the drug. After two to four minutes the dose was repeated again into the heart. After again allowing two to four minutes to elapse 0.25 c.c. of horse serum was injected into the heart.

Chloral hydrate given in this manner protects about 75 per cent of the sensitized guinea-pigs from the second injection when made directly into the heart.

CHLORAL HYDRATE INJECTED INTRACARDIACALLY FOLLOWED BY NORMAL HORSE SERUM.

Solutions of chloral hydrate were given intracardiacally in divided doses to sensitized guinea-pigs. When complete hypnosis was produced 0.25 c.c. of normal horse serum was given in the same manner.

The dilutions for injections were such that not more than 1.5 c.c. were given at one time. These injections were given very slowly so that the danger from possible dilation of the heart could be largely ruled out. Also, since intracardiac injections very frequently result in injury to the heart muscle, causing hemorrhages, and since the whole dose does not always enter the heart, experiments were repeated many times. Autopsies were performed in all cases in which death occurred in order to exclude mechanical injury with resulting hemorrhage as the cause of death.

In our preliminary work the selected dose of the drug, which was about one-half of the amount for an intramuscular dose, was given intracardiacally at one injection. This, however, frequently caused the death of the animal. We, therefore, resorted to divided doses at short intervals. With this method few deaths resulted from chloral injections.

In Table 4, guinea-pig 139 received 45 milligrams chloral hydrate intracardiacally; even before the injection was completed the animal was under the influence of the drug. The respiration was deep and irregular for about 10 seconds, then short and rapid for about one minute. Three minutes after the first injection, 40 milligrams more of the drug were given; the same irregular respirations were observed. Two minutes after the second injection of the drug, 0.25 c.c. normal horse serum was given. A few twitchings at the nose were noticed. Thirty minutes later the animal showed signs of recovery from the effects of the drug and 2½ hours later complete recovery was effected. Guinea-pig 995 received 40 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. The respiration was deep and irregular for about 10 seconds, then short and rapid. Two minutes after the first injection 40 milligrams more of the drug were given. Two minutes later 0.25 c.c. normal horse serum was given. No symptoms were noticeable. About 2½ hours later the animal had recovered from the effects of the drug.

Guinea-pig 16 received 35 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. Four minutes later 30 milligrams more of the drug were given. Three minutes later 0.25 c.c. of normal horse serum was given. No symptoms were noticeable. About two hours later the animal had recovered from the effects of the drug.

Guinea-pig 8 struggled considerably while receiving the 30 milligrams of the chloral hydrate, so that most of the drug did not enter the heart. The animal could run around immediately after the injection. Four minutes later the animal was still walking around. We then gave it 35 milligrams more of the drug in the heart this time. The animal was then under the influence of the drug. Four minutes later 0.25 c.c. normal horse serum was injected. About one-half minute later typical symptoms developed, the animal dying within four minutes. Autopsy showed no direct hemorrhage. It appears in this case as tho an insufficient amount of chloral had entered the circulation.

Guinea-pig 24 struggled considerably while receiving the 30 milligrams of chloral. Most of the drug did not enter the heart. The animal could run around. Having in mind the result of the inoculations of guinea-pig 8, we decided to wait 20 minutes before injecting again. At the end of 20 minutes the animal was in a partial stupor. Thirty milligrams of chloral were given, the animal being under the influence of the drug at the completion of the injection. Four minutes later an additional 30 milligrams of the drug were given. This was followed three minutes later with 0.25 c.c. normal horse serum. No symptoms were noticeable. About three hours later the animal recovered from the effects of the drug.

Twenty-two hours later, guinea-pigs 139, 995, 16, and 24 received an additional 0.5 c.c. normal horse serum intracardiacally. All showed typical symptoms after about one-half minute. Respiration ceased within three minutes. The heart continued to beat after respiration had ceased. Autopsies showed none of the deaths due to direct hemorrhage. These experiments were repeated many times with similar results.

The fact that those sensitized guinea-pigs, which, while under the influence of the drug, were protected from the second injection of serum, are still sensitized, leads us to the following conclusions. Certain parts of the serum necessary to cause anaphylaxis, when injected into the circulation of a sensitized pig, must be rapidly destroyed or eliminated from circulation. Or these parts may combine with cells not vitally important to the immediate life of the animal. If this be the case, the ferment or zymogen, in those protected, sensitized guinea-pigs, has not all been utilized in destroying or splitting the serum.

In other words, the animal is not in most cases vaccinated, but

TABLE 4.

INTRACARDIAC INJECTIONS OF CHLORAL HYDRATE FOLLOWED BY 0.25 C.C. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 2 TO 4 MINUTES.

Guinea-Pig No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate in Mg. Intracardiacally	Second Injection Horse Serum Intracardiacally	Symptoms	Result	Autopsy
139	380	0.73 c.c. toxin+ 1/500 c.c. antitoxic horse serum	50	1st inj. 45 mg. 2d " 40 "	0.25 c.c.	After 10 min. slight scratches at nose	Recovered	
195	340	0.73 c.c. toxin+ 1/1200 c.c. antitoxic horse serum	30	1st " 40 " 2d " 40 "	"	None	"	
16	310	0.73 c.c. toxin+ 1/100 c.c. antitoxic horse serum	50	1st " 35 " 2d " 30 "	"	"	‘	
8	335	0.73 c.c. toxin+ 1/600 c.c. antitoxic horse serum	50	1st " 30 " missed heart 2d inj. 35 mg.	"	Typical	Death within 4 min.	No hemorrhage in pericardium
24	305	0.73 c.c. toxin+ 1/450 c.c. antitoxic horse serum	30	1st " 30 " missed heart 2d inj. 30 mg. 3d " 30 "	"	None	Recovered	
13	300	0.73 c.c. toxin+ 1/500 c.c. antitoxic horse serum	50		"	Typical	Death within in 3 min.	No hemorrhage in pericardium
42	320	0.73 c.c. toxin+ 1/1250 c.c. antitoxic horse serum	30		"	"	"	"
33	360	0.73 c.c. toxin+ 1/400 c.c. antitoxic horse serum	30		"	"	"	"

TABLE 5.

INTRACARDIAC INJECTIONS OF CHLORAL HYDRATE FOLLOWED BY 0.25 C.C. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 12 TO 15 MINUTES.

Guinea-Pig No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate Intracardiacally	Second Injection Horse Serum Intracardiacally	Symptoms	Result	Autopsy
168	300	0.73 c.c. toxin+ 1/1400 c.c. antitoxic horse serum	18	1st inj. 30 mg. 2d " 30 " 3d " 30 "	0.25 c.c.	Typical	Death within 5 min.	No hemorrhage in pericardium
11	290	0.73 c.c. toxin+ 1/1700 c.c. antitoxic horse serum	18	1st inj. 30 mg. 2d " 30 " 3d " 20 "	"	"	"	"

will react with typical symptoms, followed by death, if, after recovery from the effects of the drug, an additional 1.5 c.c. of horse serum is given intracardiacally.

We wish to emphasize that from our experiments a certain optimum content of the drug in the circulation is essential to protect the animal. The drug is rapidly destroyed or eliminated from the circulation. . This we believe can be shown by allowing 12 to 15 minutes to elapse between the intracardiac injections of the drug and 12 to 15 minutes to elapse before injecting the serum. Chloral hydrate given in this manner, with this time allowance, will not protect sensitized guinea-pigs from the second injection intracardiacally.

CHLORAL HYDRATE INJECTED INTRACARDIACALLY FOLLOWED BY 0.25 C.C. NORMAL HORSE SERUM INTRACEREBRALLY.

Sensitized guinea-pigs were trephined and then given divided doses of chloral hydrate in the manner mentioned above. The time allowance between injections of the drug was from two to four minutes. One quarter c.c. of normal horse serum was then given intracerebrally after the method of Besredka. About 75 per cent of the sensitized guinea-pigs tested in this manner were protected from the second injection intracerebrally.

TABLE 6.

INTRACARDIAC INJECTIONS OF CHLORAL HYDRATE FOLLOWED BY 0.25 C.C. NORMAL HORSE SERUM INTRACEREBRALLY. TIME INTERVAL BETWEEN INJECTIONS 2 TO 4 MINUTES.

G.-P. No.	Weight in Grams	Previous Treatment	Interval in Days	Chloral Hydrate Intracardiacally	Second Injection Horse Serum Intracerebrally	Symptoms	Results
43....	400	0.73 c.c. toxin+ 1/750 c.c. antitoxic horse serum	22	1st inj. 45 mg. 2d " 45	0.25 c.c.	None	Recovered
2....	395	0.73 c.c. toxin+ 1/500 c.c. antitoxic horse serum	32	1st " 40 " 2d " 40 "	"	"	"
26....	295	0.73 c.c. toxin+ 1/400 c.c. antitoxic horse serum	37	1st " 35 " 2d " 35 "	"	"	"
7....	415	0.73 c.c. toxin+ 1/500 c.c. antitoxic horse serum	32	1st " 45 " 2d " 45 "	"	"	"
29....	275	0.73 c.c. toxin+ 1/300 c.c. antitoxic horse serum	40		"	Typical	Death within 5 min.
333....	340	"	32		"	"	"
934....	380	"	38		"	"	"

With the different methods of administering the second injection of horse serum into sensitized guinea-pigs, which were favorably under the influence of chloral hydrate, we were able to protect about 75 per cent. In our preliminary communication we stated that we believed that with improved technic in the dosage of chloral hydrate it would be possible to protect 90 per cent of all fully sensitized guinea-pigs.

Now, however, we have come to the conclusion that, if the smallest dose of serum which will just kill any animal of a given series of sensitized guinea-pigs regularly, be injected into the remainder of the animals of the same series of sensitized guinea-pigs, when properly under the influence of chloral hydrate, protection will be afforded in practically all cases.